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Plasma homocysteine, apolipoprotein E status and vascular disease in elderly patients with mental illness

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Abstract

Background: Total plasma homocysteine (tHcy) concentration is increased in elderly patients with mental illness. Also, patients with vascular disease have significantly higher plasma tHcy concentration compared with patients without vascular disease. Apolipoprotein E (apoE) status is associated with cardiovascular disease and a major genetic risk factor is inheritance of the *e4* allele. In the present study, we investigated the association between plasma tHcy and apoE status.
Methods: The relation between apoE status, plasma tHcy and vascular disease was investigated in a cohort of consecutively enrolled elderly patients with mental illness (n = 328).
Results: Plasma tHcy concentrations were increased ($p < 0.01$) in carriers of *APOE4* (13.6 μmol/L; 9.2–21.7 μmol/L) compared to non-carriers (12.4 μmol/L; 8.3–19.9 μmol/L). The proportion of patients with vascular disease was significantly ($p < 0.001$) increased among carriers (61%) compared to non-carriers (42%). An increased percentage ($p < 0.001$) of *APOE4* carriers was observed in patients with Alzheimer's disease (AD) with (71%) or without vascular disease (42%), and in patients with vascular dementia (VaD) (54%) compared to a reference group (34%).
Conclusions: Since carriers of *APOE4* showed an increased likelihood of vascular disease, these patients need more intensive control of other modifiable vascular risk factors. Furthermore, the association between plasma tHcy and the presence of *APOE4* might be attributed to an increased proportion of vascular disease in *APOE4* carriers.
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Introduction

Apolipoprotein E (apoE) plays a central role in lipid metabolism as a component of very low-density lipoproteins and chylomicrons (1). The *APOE* gene, located at chromosome 19, is polymorphic with three common alleles – *e2, e3* and *e4* (2). One genetic risk factor associated with late-onset Alzheimer's disease (AD) is inheritance of the *e4* allele (2, 3). This allele is also associated with cardiovascular disease, unlike the other two common alleles *e2* and *e3* (2–4).

Many studies have reported significantly higher total plasma homocysteine (tHcy) in elderly patients with mental illness compared with control subjects (5–8). The concentration of plasma tHcy is influenced by several factors and their interaction (9, 10). Some of these factors are age, cobalamin/folate status, renal function and the presence of vascular disease. Many clinical and epidemiological studies published during the last 20 years show that even mild hyperhomocysteinemia is associated with vascular disease (9, 11–16). Therefore, we studied elderly patients with mental illness with respect to the presence of vascular disease (5, 17–20). Briefly, our findings showed that patients with mental illness and vascular disease had significantly higher plasma tHcy concentrations compared with patients with mental illness but without vascular disease (5, 17–20), and that increased plasma tHcy was associated primarily with the presence of vascular disease and not related to a specific psychogeriatric diagnosis.

Vascular disease plays an important role in cognitive impairment in elderly patients with mental illness (21). There is a complex relation between vascular disease and dementia (22). Vascular dementia (VaD) and AD together account for the vast majority of cases of dementia. AD, the most common type of degenerative dementia, has long been considered to be a primarily degenerative brain disease. However, many patients with AD also have vascular disease contributing to brain pathology (23). An increasing number of epidemiological studies have provided further support for the suggestion that the presence of vascular disease or traditional vascular risk factors is associated with AD (24–27). VaD is considered to be the second most common cause of dementia and may result from several different types and locations of cerebrovascular lesions. The coexistence of VaD and AD may be the most common cause of dementia, i.e., mixed

dementia where both degenerative and vascular injuries are present (23, 28).

The public health burden of AD and VaD threatens to soar in the next few decades. There is a growing need to modify known risk factors for cognitive dysfunction, and one modifiable risk factor is vascular disease (21, 22). Proper control of vascular disorders may prevent or delay the onset of cognitive impairment (21, 22, 29). We have previously investigated different markers for vascular disease and their relation to the presence of vascular disease and plasma tHcy concentrations in consecutively enrolled elderly patients with mental illness (29–31). In the present study, we further extend those studies by investigating the relation between apoE status, a genetic risk marker of vascular disease, the presence of vascular disease and plasma tHcy concentrations in elderly patients with mental illness. By investigating markers for vascular disease in these patients, it might be possible to identify patients in need of more intensive control of their vascular disease and thereby prevent or delay cognitive impairment.

Materials and methods

Study populations

The present study population consisted of 328 patients consecutively enrolled (151 males and 177 females, 69 (51–83) years, median and 10th–90th percentiles) who were referred to the Psychogeriatric Department at Lund University Hospital for diagnostic assessments and treatment. Patients on any type of ongoing vitamin therapy were excluded from the study. The diagnosis was based on psychiatric, neurological, somatic, laboratory investigations, psychometric testing, measurements of regional cerebral blood flow, electroencephalography, and computer tomography scan (CT) or magnetic resonance imaging (5). The majority of cases were living in their own homes, alone or with relatives. The diagnosis of the psychogeriatric diseases was based on the DSM-IV criteria (32). Furthermore, patients with VaD fulfilled the NINDS-AIREN criteria (33) for VaD. Patients with AD were diagnosed in accordance with the NINCDS-ADRDA criteria (34). Organic dementia was diagnosed in 146 patients, 61 patients were diagnosed with AD, 11 patients with frontal lobe degeneration of non-Alzheimer type (FTD), 39 patients with VaD, 17 patients with mixed vascular and Alzheimer type dementia (MX), 13 patients with other types of dementia (Oth Dem) and five patients with dementia NUD. One hundred and eighty-two patients suffered from non-organic mental diseases, two patients had delirium (Del), 23 patients depression (Depr), 13 patients other psychiatric disorders (Oth Psych, mainly psychosis), and 85 patients had mild cognitive impairment (MCI) (35). In addition, 59 patients presented with subjective symptoms of cognitive impairment but normal psychometric tests. They had normal CT and otherwise were apparently healthy. These patients were treated as a separate group of patients called subjective cognitive impairment (SCI). The *APOE* genotypes in the different diagnostic groups are shown in Table 1. The study was approved by the Ethical Committee of the University of Lund. Informed consent to participate was given by all subjects (or relatives if the patients were unable to communicate).

The patients were divided into two groups according to the presence or absence of vascular disease. Both cerebral and extra-cerebral vascular diseases were included in the group of patients with vascular disease. The presence of vascular disease in the patients was based on diagnosis and/or symptoms indicating vascular disease as indicated in their medical records. One group (n = 162) consisted of patients with vascular disease and included diagnoses/symptoms, such as VaD, cerebral infarction, transitory ischaemic attacks, myocardial infarction, angina pectoris, peripheral vascular disease, atrial fibrillation, and hypertension. In another group of 166 patients, there was no indication of any vascular disease.

Assays

Blood samples for homocysteine determination were collected in evacuated tubes containing EDTA at about 8 a.m. following an

Table 1 Diagnoses of patients with and without vascular disease according to *APOE* status.

	Number of patients	*APOE2/2*	*APOE2/3*	*APOE2/4*	*APOE3/3*	*APOE3/4*	*APOE4/4*
Number of genotypes	328	3	37	12	150	95	31
Dementia of Alzheimer type	61		5		22	22	12
Mixed vascular and Alzheimer type dementia	17		2	2	4	7	2
Frontal lobe degeneration of non-Alzheimer type	11		2	1	4	2	2
Vascular dementia	39		3	4	15	15	2
Unspecified dementia	5				4		1
Other specified types of dementia	13		1		5	6	1
Delirium	2				2		
Depression	23	1	2	1	13	6	
Other non-organic psychiatric disorders	13		1		9	1	2
Mild cognitive impairment	85	1	11	3	44	21	5
Subjective cognitive impairment	59	1	10	1	28	15	4
No vascular disease	166	1	20	3	91	40	11
Vascular disease	162	2	17	9	59	55	20

overnight fast and centrifuged within 15 min at 3000×*g* for 5 min. Plasma tHcy was measured with HPLC after reduction of disulphide bonds with dithiothreitol and deproteinisation with sulphosalicylic acid (36). The upper reference limit (95th percentile) for plasma tHcy in a healthy elderly population without folate fortification is 19.9 μmol/L (17, 18).

APOE genotyping was performed using a method of restriction genotyping as described by Hixson (37). The percentage of *APOE4* carriers (*APOE2/E4, E3/E4* and *E4/E4*) in a healthy Swedish population is about 30% (38).

Serum cobalamin (S-Cob) and serum folate (S-Fol) concentrations were determined using competitive protein binding assays (Roche Diagnostics, Mannheim, Germany; Modular Analytics E170). For S-Cob we used the reference interval of 150–650 pmol/L (5). The reference interval according to the manufacturer (Roche Diagnostics) for S-Fol in individuals without folate fortification is 7–39 nmol/L.

Serum creatinine (S-Creat) concentrations (upper reference limit 105 μmol/L for males and 95 μmol/L for females) were measured with an enzymatic method (Roche Diagnostics; Modular Analytics P).

Serum C-reactive protein (CRP) (upper reference limit 3.0 mg/L) was measured in a subpopulation of the present study population (n = 58) with a routine method (Roche Diagnostics; Modular Analytics P).

Statistics

The results are presented as medians and 10th–90th percentiles. The following two-tailed tests at the 5% level of significance were used to evaluate the study: Kruskal-Wallis one-way analysis of variance with Bonferoni-correction in cases with three or more independent samples, Mann-Whitney U-test for two independent samples. The χ^2-test was used for comparison of frequencies between *APOE4* carriers and non-carriers (Table 3) and percentage of *APOE4* carriers in different diagnostic groups (Table 4). A general linear model (GLM) was used to identify factors with effects on plasma tHcy concentrations. Logistic regression analysis was performed in which the presence or absence of vascular disease was included as the dependent variable.

Results

The most frequent *APOE* genotype was *e3/e3* (n = 150), and *e3/e4* (n = 95), followed in descending order by *e3/e2* (n = 37), *e4/e4* (n = 31), *e4/e2* (n = 12), and *e2/e2* (n = 3). Diagnoses and patients with and without vascular disease in the different apoE status are shown in Table 1. Plasma tHcy concentrations were increased ($p < 0.001$) in patients with vascular disease (14.1; 9.1–21.2 μmol/L, n = 162) compared with patients without vascular disease (11.6; 8.5–19.3, n = 166). Plasma tHcy concentrations and age according to *APOE* status are shown in Table 2. There were no significant differences in these variables between the different *APOE* genotypes.

Table 3 Plasma tHcy, serum cobalamin (S-Cob), serum folate (S-Fol), serum creatinine (S-Creat), patients with and without vascular disease, and patients with dementia and non-dementia in *APOE4* carriers and non-carriers.

	Non-carriers	Carriers
Number	190	138
P-tHcy, μmol/L	12.4 (8.3–9.9)	13.6 (9.2–21.7)[b]
S-Cob, pmol/L	307 (183–468)	291 (177–486)
S-Fol, nmol/L	13.0 (7.5–25.3)	14.0 (8.2–22)
S-Creat, μmol/L	75 (58–108)	78 (57–114)
Vascular disease, n	78 (41%)	84 (61%)[a]
No vascular disease, n	112 (59%)	54 (39%)[a]
Dementia, n	67 (35%)	79 (57%)[b]
Non-dementia, n	123 (65%)	59 (43%)[b]

[a]$p < 0.001$; [b]$p < 0.01$. Non-carriers are compared to carriers. Median, 10th–90th percentiles are presented for plasma tHcy, S-Cob, S-Fol and S-Creat, whereas number and percentage are presented for the patients.

Table 3 shows a comparison of *APOE4* carriers to non-carriers. Plasma tHcy concentrations were increased in carriers compared to non-carriers. Age correction of plasma tHcy did not change the significant difference. There was no significant difference between carriers and non-carriers with respect to use of drugs that may affect plasma tHcy concentrations such as folate, cobalamin or vitamin B6 antagonists. No association with *APOE4* status was observed with respect to S-Creat, S-Cob or S-Fol. The proportion of patients with vascular disease was significantly increased among carriers compared to non-carriers, whereas patients without vascular disease exhibited a significantly increased proportion among non-carriers compared to carriers. Likewise, patients with dementia exhibited a significantly higher proportion among carriers compared to non-carriers, and patients without dementia exhibited a significantly increased proportion among non-carriers compared to carriers.

In Table 4, the percentage of *APOE4* carriers is presented in some diagnostic groups and compared to patients with SCI, since these patients exhibited a normal percentage of *APOE4* carriers. Patients with AD, with or without vascular disease, showed a significantly increased percentage of *APOE4* carriers compared to patients with SCI. Patients with AD and vascular disease also showed an increased percentage of *APOE4* carriers ($p > 0.001$) compared to AD patients

Table 2 Plasma tHcy concentration and age according to *APOE* status.

	APOE2/2	*APOE2/3*	*APOE2/4*	*APOE3/3*	*APOE3/4*	*APOE4/4*
Number of genotypes	3	37	12	150	95	31
P-tHcy, μmol/L	13.5 (5.8–16.2)	12.8 (8.8–20.1)	12.9 (8.5–24.1)	12.4 (8.3–20)	13.9 (9.3–22.5)	13.1 (9.1–20)
Age, years	73 (60–86)	68 (49–84)	72 (55–83)	64 (49–84)	72 (53–83)	71 (53–81)

Median, 10th–90th percentiles are presented.

Table 4 Age, plasma tHcy, and percentage of *APOE4* carriers in different diagnostic groups.

	Number	Age, years	P-tHcy, μmol/L	*APOE4* carriers, %
AD	61	72 (54–87)[a]	15.0 (10.3–21.6)[a]	56[a]
AD with vascular disease	28	72 (61–84)[a]	15.1 (10.7–20.5)[b]	71[a]
AD without vascular disease	33	71 (52–90)[a]	14.5 (10–22.8)[c]	42[a]
MCI	85	62 (50–77)[c]	11.7 (8.8–17.3)[c]	34
MCI with vascular disease	40	69 (54–78)[a]	12.5 (9–21.5)[c]	37
MCI without vascular disease	45	57 (47–72)	11.3 (8.4–16.1)	31
VaD	39	80 (69–87)[a]	17.0 (9.5–25.7)[a]	54[a]
Depr	29	66 (48–84)[c]	11.4 (7.4–16.7)	30
SCI	59	57 (41–73)	10.7 (7.8–15.7)	34

[a]$p < 0.001$; [b]$p < 0.01$; [c]$p < 0.05$. The different diagnostic groups are compared with patients with SCI. Kruskal-Wallis and Mann-Whitney tests were used to detect significant changes in plasma tHcy levels and age. Median, 10th–90th percentiles are presented for age and plasma tHcy, whereas percentages are presented for *APOE4* carriers.

without vascular disease. Patients with MCI, with or without vascular disease, exhibited no significant increase in carriers compared to patients with SCI, whereas patients with VaD showed an increased percentage of *APOE4* carriers compared to patients with SCI.

Plasma tHcy concentrations were increased in AD patients with or without vascular disease, in MCI patients with vascular disease, and in patients with VaD compared to patients with SCI (Table 4). All diagnostic groups, except MCI without vascular disease, exhibited older age than patients with SCI. In addition, there were no significant differences between S-Cob, S-Fol or S-Creat among the different diagnostic groups shown in Table 4 (data not shown).

In a subgroup that included 58 randomly selected patients, 43 had normal serum CRP and 15 had increased serum CRP (above 3 mg/L). Carriers of *APOE4* with both normal and increased serum CRP showed no significant changes in plasma tHcy concentrations compared to non-carriers (normal CRP: carriers 12.5, 7.9–23.7 μmol/L, $n = 20$ and non-carriers 10.4, 7.1–22.7, $n = 23$; increased CRP: carriers 13.3, 6.3–16.7 μmol/L, $n = 8$ and non-carriers 9.0, 6.7–16.0, $n = 8$).

Multivariate analysis (GLM) that included age, S-Fol, S-Cob, S-Creat, and *APOE4* status showed that age, S-Fol, S-Creat and S-Cob significantly predicted plasma tHcy concentrations (Table 5). Logistic regression analysis in all patients, with the presence or absence of vascular disease as the dependent variable, showed that plasma tHcy, adjusted for its significant predictors (age, S-Fol, S-Creat and S-Cob), and the presence of *APOE4* status were significant predictors of vascular disease (adjusted plasma tHcy – t-value 3.413, $p < 0.001$; presence of *APOE4* status – t-value 2.959, $p < 0.01$).

Table 5 General linear model estimates of factors with potential impact on plasma tHcy concentrations (dependent variable).

Independent variable	t-Value	p-Value
Age	5.588	<0.001
S-Fol	–5.293	<0.001
S-Cob	–2.079	0.039
S-Creat	6.259	<0.001
Presence of *APOE4* status (yes/no)	–0.193	0.847

Discussion

The present study population consisted of consecutively enrolled patients with mental illness. Approximately half of the patients had co-existent vascular disease and ~40% had dementia. The current study showed that in these elderly patients with mental illness, plasma tHcy concentrations were increased in *APOE4* carriers compared to non-carriers. In previous investigations (39–41), no association was observed between plasma tHcy and *APOE4* status. Those investigations, however, did not focus specifically on the association of interest, and two (39, 40) had small population sizes. In a recent study (42), *APOE4* carriers in an elderly healthy population had an even lower risk of hyperhomocysteinemia than did non-carriers, but only if the carriers did not have increased CRP concentrations. The authors (42) suggested that this finding could possibly be attributed to CRP and plasma tHcy competing for the same mechanism (perhaps a lipoprotein carrier) that favours their clearance from blood in *APOE4* carriers. However, in a small subpopulation of the present study population we could observe no such association between *APOE4* carrier status and concentrations of plasma tHcy and serum CRP. Carriers of *APOE4* with both normal and increased serum CRP showed a tendency towards increased plasma tHcy (not significant) compared to non-carriers. The discrepant findings might possibly be explained by different selection criteria and different populations. In the present study, patients with vascular disease showed an increased proportion among *APOE4* carriers, which is in agreement with previous studies (2–4). The increased proportion of patients with vascular disease in *APOE4* carriers could explain the increased plasma tHcy concentrations in carriers, since the presence of vascular disease is associated with increased concentrations of plasma tHcy (9, 11–16).

Clarification of the role of vascular risk factors for cognitive dysfunction is important since these may serve as targets for strategies for prevention (21, 22). Few studies have examined the effect of vascular risk factors on dementia progression after establishing a diagnosis of AD. Mielke et al. (43) have shown that atrial fibrillation, hypertension and angina pectoris were associated with a greater rate of decline and thus may represent modifiable risk factors for secondary prevention in AD. Likewise, Song et al. (44) observed that AD with silent cerebral infarction showed a more severe cognitive decline than AD without vascular disease, indicating that cerebrovascular disease contributes to the severity of cognitive decline. Also, Sheng et al. (45) showed that AD with coexisting cerebral infarction (satisfying criteria for VaD) was associated with faster progression of dementia. These findings suggest that prevention of cerebrovascular disease may play an important role in preventing the rapid cognitive decline of AD.

The percentage of *APOE4* carriers in normal subjects has been reported to be about 30% (38). An increased percentage of *APOE4* carriers was observed in AD patients with or without vascular disease, which is in agreement with previous studies (2, 3). In addition, AD patients with vascular disease exhibited a higher percentage (71%) of *APOE4* carriers than AD patients without vascular disease (42%). In previous investigations, plasma tHcy concentrations were also observed to be increased in AD patients with vascular disease compared to AD patients without vascular disease (20, 29). ApoE4 might contribute to AD pathology by interacting with multiple factors through various pathways (46). For example, interactions with the amyloid β-peptide and the amyloid cascade may lead to cognitive decline and neurodegeneration. However, since patients with AD and vascular disease showed a higher percentage of *APOE4* carriers than patients with AD without vascular disease, it is possible that apoE4 primarily reflects the presence of vascular disease. In agreement with the findings of Religa et al. (39), patients with MCI did not show an increased percentage of *APOE4* carriers. Most previous studies have also demonstrated an association between *APOE4* carriers and patients with VaD (47). Although not all studies found a significant association, some studies (47) that did not find a significant association did find a tendency towards a greater frequency of *APOE4* carriers in VaD. In the present study, we observed a significant increase in carriers. However, the number of patients with VaD was relatively small.

APOE4 carriers have been associated with cognitive decline in elderly subjects (41, 48). In accordance with these findings, we observed an increased frequency of patients with dementia in the group of *APOE4* carriers compared to non-carriers. One possible reason for the association of the *APOE4* genotype and cognitive impairment is that the *APOE4* genotype is related to vascular disease which, in turn, is related to cognitive impairment (21).

In the present study, we have investigated *APOE4* status as a possible risk marker for vascular disease in elderly patients with mental illness. *APOE4* carriers showed an increased proportion in patients with vascular disease. Likewise, the presence of *APOE4* status predicted the presence of vascular disease. The presence of *APOE4* status in patients with mental illness might therefore be useful to identify patients at risk of rapid progression of vascular disease and consequently also of cognitive decline. Therefore, carriers of *APOE4* might require more intensive control of other modifiable vascular risk factors, such as blood pressure or cholesterol. Furthermore, the association between plasma tHcy and the presence of apoE4 might be attributed to an increased proportion of vascular disease in *APOE4* carriers.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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